Protocol for genotyping iCre transgenic rats

NIDA Optogenetics and Transgenic Technology Core (OTTC)

March 14, 2017

Prepare genomic DNA using the HotShot Method:

This method was adapted by NIDA-OTTC from the protocol originally described in *Biotechniques 29(1), 52-54 (2000)*. It has been used successfully with ear punches and tail clips of rats.

Place the biopsy sample in 1.5ml microfuge tube.
Add 200 microliters of 50mM NaOH
Incubate tubes at 95C for 60 minutes.
Vortex tubes on medium power setting for 5 seconds.
Quick spin the tubes to bring down the condensation.
Neutralize each sample by adding 20 microliters of 1M Tris-HCl (pH 8).
Vortex tubes on medium power setting for 5 seconds.
Quick spin the tubes to bring down the condensation.

Debris (the "undigested" remnant of sample) may remain visible at the bottom of the tube. This is OK, but be sure to take only from the supernatant when setting the PCR reaction.

Determining iCre carrier/non-carrier rats by PCR genotyping:
There will be two separate reactions per sample. A transgene-specific reaction to detect iCre, and a Rosa26 reaction to serve as a positive control for your lysate quality and reaction conditions.

General assay for amplifying the iCre transgene:

Oligo Name	Sequence (5' to 3')
iCRE F738	GTTCTGCCGGGTCAGAAAGAATGGT
bGHpolyA R30	GGCTGGCAACTAGAAGGCAC

These oligos produce a 367 basepair amplicon.
These oligos have been tested using an annealing gradient and they work best between 64C and 68C.
This allows the use of the two-step PCR program described below (1taq_68, YZ026).

Positive control assay for amplifying the rat Rosa26 locus:

Oligo Name	Sequence (5' to 3')
rRosa26 F81487	CTTCAGCCACATGGTGGGTC
rRosa26 R82307	TTGGCTAACTTACCAGTTATGCTACCT

These oligos produce an 821 basepair amplicon.
These oligos have been tested using an annealing gradient and they work well between 61C and 68C.
This allows the use of the two-step PCR program described below (1taq_68).

Prepare PCR reaction mix:

Master Mix Matrix	**1 reaction** (11 μL)
2x One Taq mastermix	5.5
10x primers (For & Rev oligos, 5 μM each)	1.1
Water	3.4
Total	10

Dispense 10 μL per tube, then add 1 μL of genomic DNA lysate.

This protocol was updated on 03-14-2017 by Chris Richie.

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PCR program = 1taq_68

0. 94C – HOLD (hot start)
1. 94C – 2 min
2. 94C – 30 sec
3. 68C – 1 min
4. go to step 2, repeat 40x
5. 4C – HOLD (end of program)

Analyze PCR products

Run the samples on a 2% agarose gel in 1xTAE buffer.

Reagents List:

10M NaOH (Sigma)
1M Tris-HCl (pH 8) (Sigma-Aldrich)
OneTaq DNA polymerase mastermix (New England Biolabs)
Oligos (IDT DNA Technologies, standard synthesis and desalt preparation).

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